Saskatchewan Agriculture, Food and Rural Revitalization

ADF

AGRICULTURE DEVELOPMENT FUND

FINAL REPORT

20020157

INVESTIGATION INTO THE POPULATION STRUCTURE OF ASCOCHYTA LENTIS

Funded by: The Agriculture Development Fund

March, 2005
Prepared by: University of Saskatchewan

105-04398/

Investigation into the population structure of *Ascochyta lentis*

Final Report:

Project: 20020157 (ADF)

#AGR0302 (SPCDB)

Res.: 03AC (ADF)

March 2005

S. Banniza & A. Vandenberg

CDC

University of Saskatchewan

51 Campus Drive

Saskatoon, SK S7N 0k1

Tel (306) 653 6816

E-mail: sabine.banniza@usask.ca

Abstract

Isolates of A. lenis were obtained from 1978 to 1985 and from 2001 to 2004 for a comparative study of aggressiveness and molecular diversity. A total of 65 isolates were tested on 16 lentil genotypes that potentially contain different sources of resistance. Thirty-one isolates originated from pre-1986 and 34 isolates came from recent collections. On average, isolates from post 2000 caused more disease on lentil than those from pre1986 with the exception of Indianhead and Richlea. The magnitude of the difference in disease caused by the two populations varied. Largest differences were seen on 1156217a, ILL 1704, ILL 4605 and PI 468901. Significant effects of lentil genotypes, isolates and the interaction between both factors were observed and contributions of isolates, genotypes and the interaction were comparable suggesting that ranking of lentil genotypes based on resistance differed among isolates.

Comparing the reaction of each isolate on all lentil genotypes also showed a more or less pronounced continuum in the amount of disease caused. Indianhead and CDC Redberry showed high levels of resistance to almost all isolates of *A. lentis* whereas ILL5588 appeared to be susceptible to a large number of isolates. Three isolates were identified that appeared to show differential reaction between Indianhead and ILL5588. Aggressiveness in *A. lentis* appears to be a quantitative trait. However, some cross-over reactions of isolates with high aggressiveness on IL5588 and low on Indianhead, and *vice versa*, indicate that major genes may be involved that explain a larger amount of the variation, in addition to many minor genes. The same situation appears to be present in lentil genotype resistance. Molecular analysis of isolates revealed high levels of similarity (> 80 %) among isolates collected more than 20 years apart.

(a) Executive Summary

Ascochyta lentis has been a major pathogen in lentil in Saskatchewan for several decades. Breeding for resistance to the disease is a major objective in the lentil breeding program of the Crop Development Centre. Some progress has been made in recent years in developing varieties with higher levels of resistance.

A total of 65 isolates of *A. lentis* was evaluated in 9 tests on 16 lentil genotypes that were assumed to possess different levels of resistance and different resistance genes to the pathogen. Thirty-one isolates originated from 1978 to 1985 and 34 isolates had been collected primarily in 2003. Disease was measured as area under the disease progress curve and analyzed using non-parametric statistics. Results of pathogenicity testing suggest that on average isolates from 2003 are more aggressive compared to isolates collected in 1985 and before. Some deviation from this trend was obvious on some lentil genotypes. No increase in aggressiveness in the post 2000 population was observed on Indianhead and Richlea whereas largest differences were seen on 1156217a, ILL 1704, ILL 4605 and PI 468901. Richlea is one of the most susceptible varieties to *A. lentis* and it can be assumed that it therefore does not impose a large degree of selection pressure on the fungal population. Little changes on Indianhead suggest that the resistance in this variety has not been 'broken' by new biotypes of the fungus.

Analysis of data revealed significant differences between isolates, between genotypes and a significant genotype x isolate interaction. Partitioning of the total variation between isolates, genotypes and the genotype x isolate interaction revealed isolates contributed between 6 and 24 % to the total variation, genotypes between 21 and 41 %, and the interaction 16 and 29 %. The amount of variation explained through isolates, genotypes and the interaction ranged from 60 to 80% suggesting that environmental factors have a large effect on disease development, even under controlled conditions as in these experiments. To investigate this interaction further, the distributions of disease reactions of isolates on each lentil genotype, and of each isolates on all lentil genotypes were visually analyzed further.

Plotting relative disease of isolates for each lentil genotype showed that isolates induced in large parts a continuum of aggressiveness, rather than distinct groups. Single isolates of both populations caused distinctly more disease on some lentil genotypes compared to most other isolates. Many isolates were able to infect ILL5588 to a significant level. However, one isolate from 1985 was identified that caused very little disease on ILL5588 and high disease on Indianhead, therefore may be used as a differential for the different sources of resistance found in ILL5588 and Indianhead. Another two isolates from 2003 and 2004, respectively, showed the opposite reaction on those two lentil genotypes and could be used for the same purpose. One isolate from 2003 was the only isolate that caused significant amount of disease on CDC Robin.

Molecular analysis revealed that all isolates were relatively similar sharing more than 80% of bands/loci revealed by 8 RAPD primers. Cluster analysis depicted three groups among the isolates. One cluster consisted of almost exclusively isolates from pre 1986 with similarities between 90 to 98 %. One isolate in this cluster had been collected in 2003. Two pre-1986 isolates, collected 4 years apart were identical in all banding patterns suggesting that some genotypes persist in the population for several years.

A second group of isolates from pre-1986 with similarities ranging from 93 to 98 % was more similar to a third cluster consisting exclusively of isolates from post 2000 at 89 % than to the first cluster of pre-1986 isolates, with which it shared 84 % similarity. In the group of isolates from post 2000, two groups of isolates were present with identical banding patterns for all 8 primers. These isolates came from infected seed of various locations in Saskatchewan, but originated from the same area.

Results from this study suggest that aggressiveness in the isolates is a quantitative trait indicating that there is no distinct race structure in the pathogen population. However, some cross-over reactions of isolates with high aggressiveness on IL5588 and low on Indianhead, and *vice versa*, indicate that major genes may be involved that explain a larger amount of the variation, in addition to many minor genes. The same situation appears to be present in lentil genotype resistance. Molecular analysis of isolates by RAPD-PCR revealed relatively little polymorphism suggesting that the population of *A. lentis* in Saskatchewan has not been very diverse genetically.

(b) Technical Report

Background:

Ascochyta lentis has been a major pathogen in lentil in Saskatchewan for several decades. Breeding for resistance to the disease is a major objective in the lentil breeding program of the Crop Development Centre. Some progress has been made in recent years in developing varieties with higher levels of resistance. Until recently it was assumed that there is no cultivar specialization among isolates of the fungus. However, results from investigations into the virulence of isolates of the pathogen in Australia, including one Canadian isolate, showed that this Canadian isolate caused severe infection on ILL 5588, but was only weakly virulent on Indianhead (R. Ford, pers. com.). These two accessions represent the major sources of resistance currently in use. Differential reaction of this Canadian isolate indicates that we may witness a process of cultivar specialization, i.e. the development of pathotypes in the W-Canadian population of *A. lentis*. Previous investigations into the population structure of *A. lentis* in W-Canada (Ahmed *et al.*, 1996) showed that the fungus is able to change in virulence in a relatively short period of time. Although cultivar specialization was not present in 1992, an increase in use of cultivars with different sources of resistance during the past decade may have led to the co-evolution of a more diverse and specialized fungal population.

Project Objectives:

The objectives of this project are to investigate the diversity and virulence of current isolates of *A. lentis* found in Saskatchewan lentil fields by pathogenicity testing and RAPD-PCR. Isolates from post 2000 were compared with those from pre 1986 (Ahmed *et al.*, 1996) to test whether the population of current isolates has increased in aggressiveness.

Materials and Methods:

Establishment of a culture collection:

Isolates were obtained from infected lentil seed. Seed was surface sterilized in 10% bleach solution and plated out on tab water agar. Growing colonies identified as *Ascochyta lentis* were transferred onto potato dextrose agar. Single spores of these colonies were re-isolated and grown into single spore cultures. Because some infected seed had been in storage at -20C for more than 10 years, isolates from this seed was inoculated onto a susceptible lentil cultivar and re-isolated to ensure that all pathogenicity genes were 'switched on'. A total of 64 isolates were tested for pathogenicity testing, including 31 originated from 1978 to 1985 (partly used in Ahmed *et al.*, 1996), 1 isolate from 2001, 29 from 2003 and 4 isolates from 2004 (Table 1). Isolates are stored in cryo-preservation for future studies.

Pathogenicity testing:

Sixteen lentil genotypes (964a-46, 1156-2-17a, 1254s-1, ILL 1704, ILL 4605, ILL 5588, ILL 5684, ILL 7537, PI 320937, PI 339283 , PI 345629, PI 468901, Eston, Indianhead, Richlea, and Robin) were seeded into 4-inch square pots filled with Redi-Earth at 6 seeds per pot and thinned to 4 plants after germination. The plants were grown in greenhouse conditions with day/night temperature of 23°C/18°C and 16hrs of light and fertilized with 20-20-20 soluble fertilizer at 1week after germination. Plastic sheets were wrapped around pots to segregate treatments and facilitate moisture retention. Plants were inoculated 2 weeks after seeding with spore suspensions of isolates of *Ascochyta lentis* at a concentration of 5×10^5 spores mL^{-1}. Plants were inoculated with an airbrush connected to a CO_2 cylinder delivering approximately 1.5mls spore suspension per plant at 20 p.s.i., were incubated in a humidity chamber for 48 hours and then moved to a mist bench with same temperature/light conditions as above for the remainder of the test. A misting irrigation misted the plants every 30 minutes for 30 second durations from 7am to 11pm daily. The experiment was designed as a completely randomized design with 4 replications. Plants were rated for disease at day 3, 8, 13 and 17 after inoculation using the Horsefall-Barratt scale. The area under the disease progress curve (AUDPC) was calculated for each isolate/genotype combination. As the number of isolates and varieties exceeded the reasonable size for one experiment, isolates were tested in groups of 5 to 9 isolates on 16 sixteen lentil genotypes. In order to compare reactions between isolates, a 'standard' isolate (AL60901 from 2001) was included in each test and reactions of other isolates were expressed as proportion of the standard for comparisons between tests.

ANOVA was performed on the AUDPC data to determine the proportion of variability contributed by isolates, variety and the interaction of isolates by variety. Because data had heterogeneous variance that could not be stabilized by transformation, significance levels of factors were determined using a non-parametric approach as described by Shah and Madden (2004).

DNA extraction and RAPD-PCR analysis:

Isolates were grown on 10 mL glucose-yeast medium (1.0 g $NH_4H_2PO_4$, 0.2 g KCl, 0.2 g $MgSO_4.7H_2O$, 10.0 g glucose, 5.0 g yeast extract, 1.0 mL 0.5% (w/v) Copper solution, 1.0 mL 1% (w/v) Zinc solution,

$1L^{-1}$ distilled H_20) and incubated in a shaking incubator at 22°C/120rpm with 24hr light for 72hrs before being aseptically transferred to 250ml Erlenmyer flasks containing 100 mL glucose-yeast medium. Cultures were incubated for a further 4-7days. Mycelium was harvested by vacuum filtration under sterile conditions and freeze dried. Prior to extraction, mycelium was pulverized.

DNA extraction was conducted following the method described by Raeder and Broda (1985). An amount of 50 mg of pulverized mycelium was placed into a micro-centrifuge tube, mixed and incubated with 500ul extraction buffer (0.15M NaCl, 50mM Tris-HCL, 10mM EDTA, 2 % sodium dodecyl sulphate, pH8). To this, 250 µl phenol and chloroform each was added, gently mixed and centrifuged in 4°C at 8,500g for 1 hour. The upper aqueous layer was removed, incubated with 25 µl of RNAse (20 mg ribonuclease A/mL TE buffer) for 10 min, after which an equal volume of chloroform:isoamyl alcohol (24:1) was added. After mixing, tubes were centrifuged for 10 min at 8,500g, and the upper aqueous layer was recovered. DNA was precipitated by adding 0.54 times the volume of cold isopropanol. Pellets were washed in 70 % ethanol and redissolved in 10 to 20 µL TE buffer.

For PCR, reactions of 20µL were set up using 40ng of DNA , 0.6 units of *Taq* DNA polymerase, 0.24 mmol of each dNTP, 0.3 µmolor primer and PCR buffer.

Among 20 primers tested to date, 8 produced polymorphic banding patterns and have been used to screen 34 isolates. Banding patterns were evaluated by scoring presence and absence of bands and data matrices were analyzed using DICE's genetic similarity coefficient in SIMQUAL. The resulting similarity data was assessed and grouped by cluster analysis using Unweighted Pair Group Method with Arithmatic Mean (UPGMA). All analyses were performed using NTSYS-PC.

Results and Discussion:

Pathogenicity testing:

Extensive pathogenicity testing was conducted to compare the reaction of 65 isolates of *A. lentis* on 16 lentil genotypes. Due to the high amount of isolates and differentials resulting in 4,160 experimental units, isolates were tested in batches of 5 to 9 isolates at a time. This resulted in some limitations in how data could be analyzed and what statistical comparison could be made. Whereas comparisons of isolates within the same tests were statistically possible, comparisons between tests could not be statistically verified. For trend comparison of isolates between tests, performance of each isolate on each differential was related to the standard isolate AL60901 that was included in each test to compensate for test-specific error terms. While isolates of 2003 were collected and purified, 'old' isolates from pre 1986 were already tested, resulting in isolates from pre 1986 and post 2000 being tested in separate tests in most cases. An alternative approach would have been to use an incomplete experimental design, but that would have required all isolates to be available (i.e. in pure culture) prior to starting the pathogenicity testing thereby requiring another year for this project to be completed.

Comparing isolates from pre 1986 and post 2000, measured as relative to the standard isolate AL60901 from 2001, showed that on average isolates from post 2000 appeared to be more aggressive compared to isolates from pre 1986 (Fig. 1). Isolates from 1992 tested by Ahmed et al. (1996) showed that the population from that year was also more virulent than isolates from 1985 and before. Unfortunately, none of the 1992 isolates could be revived to date, so comparison of 2003 isolates with that population was not possible to show whether isolates have changed again during the past 10 years.

Comparison of the pre-1986 and post-2000 populations on each lentil genotype showed that a trend of increasing aggressiveness was obvious on almost all genotypes tested with the exception of Indianhead and Richlea. Richlea is one of the most susceptible varieties to *A. lentis* and it can be assumed that it

therefore does not impose a large degree of selection pressure on the fungal population. Little changes on Indianhead suggest that the resistance in this variety has not been 'broken' by new biotypes of the fungus. Considering that Indianhead is a valuable source of resistance in newer lentil varieties, this is an important finding. The magnitude of the difference in disease caused by the two populations on other lentil genotypes varied. Largest differences were seen on 1156217a, ILL 1704, ILL 4605 and PI 468901. Reaction of various breeding lines has to be evaluated in context of the resistance breeding program and may suggest that some lines should be excluded from the crossing program because of increasing virulence of isolates on that material.

Analysis of data revealed significant differences between isolates, between genotypes and a significant genotype x isolate interaction. Partitioning of the total variation between isolates, genotypes and the genotype x isolate interaction revealed isolates contributed between 6 and 24 % to the total variation, genotypes between 21 and 41 %, and the interaction 16 and 29 % (Table 2). The amount of variation explained through isolates, genotypes and the interaction ranged from 60 to 80% suggesting that environmental factors have a relatively large effect on disease development, even under controlled conditions as in these experiments. The impact on environmental factors on disease development is a major constraint to reliably testing germplasm for resistance. We have been continuously improving screening conditions by standardizing all aspects of the screening process with the objective of minimizing the impact of environmental factors on disease development, but some diseases such as the ascochyta blights on pulses appear to be inherently sensitive to small changes in some factors beyond our control.

Contributions of isolates, genotypes and the interaction were comparable suggesting that ranking of lentil genotypes based on resistance differed among isolates to some degree. To investigate this interaction further, the distributions of disease reactions of isolates on each lentil genotype, and of each isolates on all lentil genotypes were visually analyzed further.

Plotting relative disease of isolates for each lentil genotype demonstrated that isolates showed in large parts a continuum of aggressiveness, rather than distinct groups (Fig. 2). Single isolates of both populations caused distinctly more disease on some lentil genotypes compared to most other isolates. For example, isolate AL281203 isolated in 2003 cause significantly more disease compared to other isolates of that year on CDC Robin; isolate AL131203 from 2003 was significantly more aggressive on 115617a; AL510104 from 1985 caused more disease on 964a46 and AL281203 from 2003 on ILL5588 (Fig. 2).

Comparing the reaction of each isolate on all lentil genotypes also showed a more or less pronounced continuum in the amount of disease caused, though isolates differed in the overall amount of aggressiveness, and in the specific response of lentil genotypes. Indianhead and CDC Redberry showed high levels of resistance to almost all isolates of *A. lentis*. Two isolates from pre 1986, AL370104 and AL260104 induced high amounts of disease on Indianhead (Fig. 3). Many isolates were able to infect ILL5588 to a significant level (e.g. AL131203, Fig. 3). At the same time, AL260104 caused very little disease on ILL5588 and may be used as a differential for the different sources of resistance found in ILL5588 and Indianhead. AL411203 from 2003 and AL41104 from 2004 showed high aggressiveness on ILL5588 and little on Indianhead. AL281204 from 2003 was the only isolate that caused significant amount of disease on CDC Robin (Fig. 3).

Results from pathogenicity testing suggest that the population of isolates from post 2000 is more aggressive on most lentil genotypes tested. The fact that disease reactions of isolates represent continia rather than delineating distinct groups suggests that the population of *A. lentis* is not characterized by distinct races. Aggressiveness in the isolates appears to be a quantitative trait although some cross-over reactions of isolates with high aggressiveness on IL5588 and low on Indianhead, and *vice versa*, indicate that major genes may be involved that explain a larger amount of the variation, in addition to many minor genes. The same situation appears to be present in lentil genotype resistance.

The appearance of an isolate with high aggressiveness on CDC Robin is worrying and has to be confirmed by retesting. Similarly, disease reactions of many isolates on ILL5588 causing high levels of disease suggest that the resistance gene(s) in this line may not be very effective anymore in controlling the pathogen.

RAPD-PCR analysis

Among 20 RAPD primers tested to date, 8 have been identified to date producing polymorphisms in isolates of *A. lentis* and were used for molecular analysis (OP M18, OP Y03, OP X10, OP X11, OP M03, OP G10, OP G16, OP V10). A total of 38 loci were detected with these primers, 17 of which were polymorphic. An example is given in Figure 4 for OP Y03.

Analysis of molecular data revealed that isolates of *A. lentis* from all years had more than 80 % similarity based on the 17 polymorphic loci evaluated to date, regardless of the year of collection (Fig. 5). Although the number of loci sampled to date is very limited, this suggests that isolates have not diverged significantly over a period of approximately 20 years.

One cluster consisted of almost exclusively isolates from pre 1986 with similarities between 90 to 100 %. One isolate in this cluster, AL151203 had been collected in 2003. Two pre-1986 were identical in all banding patterns, AL170104 collected in 1983 and AL450104 collected in 1979. This suggests that some genotypes can persist in the population for several years.

A second group of isolates from pre-1986 with similarities ranging from 93 to 98 % was more similar to a third cluster consisting exclusively of isolates from post 2000 at 89 % than to the first cluster of pre-1986 isolates, with which it shared 84 % similarity. In the group of isolates from post 2000, two groups of isolates with identical banding patterns for all 8 primers were present. Most of these isolates came from infected seed of various locations in Saskatchewan suggesting that the same (or very similar) genotypes are spread throughout the province. Two of these isolates originated from the same area, AL271203 and AL311203 both from Landis.

More primers and isolates will be tested to evaluate the genetic diversity of isolates of *A. lentis* in Saskatchewan, thereby establishing benchmark data for future studies to monitor the evolution of the population of this pathogen.

References:

Ahmed, S., Morrall, R.A.A., and J.W. Sheard 1996: Virulence of *Ascochyta fabae* f.sp. *lentis* on lentil. Canadian Journal of Plant Pathology 18:354-361.

Raeder, U. and P. Broda 1985: Rapid preparation of DNA from filamentous fungi. Letters of Applied Microbiology 1:17-20.

Schah, D.A., and L.V. Madden 2004: Nonparametric analysis of ordinal data in designed factorial experiments. Phytopathology 94:33-43.

Table 1: List of isolates of *Asochyta lentis* used in this study

Isolates ID	Year of collection	RM #	Location	pathogenicity	DNA
AL 2-0104	1978	unknown	unknown	Yes	Yes
AL17-0104	1983	unknown	unknown	Yes	Yes
AL 18-0104	1978	unknown	unknown	Yes	Yes
AL 22-0104	1980	unknown	unknown	Yes	Yes
AL 23-0104	1985	unknown	unknown	Yes	Yes
AL 24-0104	1980	unknown	unknown	Yes	Yes
AL 26-0104	1985	unknown	unknown	Yes	
AL 28-0104	1985	unknown	unknown	Yes	Yes
AL 30-0104	1982	unknown	unknown	Yes	Yes
AL 31-0104	1982	unknown	unknown	Yes	Yes
AL 32-0104	1980	unknown	unknown	Yes	Yes
AL 33-0104	1985	unknown	unknown	Yes	Yes
AL 34-0104	1985	unknown	unknown	Yes	
AL 35-0104	1980	unknown	unknown	Yes	Yes
AL 36-0104	1980	unknown	unknown	Yes	Yes
AL 37-0104	1980	unknown	unknown	Yes	
AL 87-0104	1985	unknown	unknown		
AL 39-0104	1980	unknown	unknown	Yes	
AL 40-0104	1985	unknown	unknown	Yes	
AL 41-0104	1979	unknown	unknown	Yes	Yes
AL 42-0104	1982	unknown	unknown	Yes	
AL 43-0104	1980	unknown	unknown	Yes	Yes
AL 44-0104	1981	unknown	unknown	Yes	
AL 45-0104	1979	unknown	unknown	Yes	Yes
AL 46-0104	1980	unknown	unknown	Yes	
AL 47-0104	1985	unknown	unknown	Yes	
AL 48-0104	1983	unknown	unknown	Yes	Yes
AL 49-0104	1983	unknown	unknown	Yes	
AL 50-0104	1980	unknown	unknown	Yes	
AL 51-0104	1985	unknown	unknown	Yes	
AL 52-0104	1985	unknown	unknown	Yes	
AL 53-0104	1985	unknown	unknown	Yes	
AL 1-1203	2003	RM 111972	Sedley, Sk		
AL 2-1203	2003	RM 112084	Kenaston, Sk		
AL 3-1203	2003	RM 112347	Rosetown, Sk	Yes	Yes
AL 4-1203	2003	RM 112348	Rosetown, Sk		
AL 5-1203	2003	RM 112385	Perdue, Sk		
AL 6-1203	2003	RM 112398	Swift Current, Sk		
AL 7-1203	2003	RM 112404	Rosetown, Sk	Yes	Yes
AL 8-1203	2003	RM 112423	Delisle, Sk	Yes	
AL 9-1203	2003	RM 112782	Lumsden, Sk	Yes	
AL 10-1203	2003	RM 113144	Saskatoon, Sk		
AL 11-1203	2003	RM 113363	Allan, Sk		
AL 12-1203	2003	RM 113487	Herschel, Sk		

Isolates ID	Year of collection	RM #	Location	pathogenicity	DNA
AL 13-1203	2003	RM 113506	Wymark, Sk	Yes	
AL 14-1203	2003	RM 113508	Swift Current, Sk	Yes	Yes
AL 15-1203	2003	RM 113524	Holdfast, Sk	Yes	Yes
AL 16-1203	2003	RM 113548	Lancer, Sk		
AL 17-1203	2003	RM 113647	Handel, Sk	Yes	Yes
AL 18-1203	2003	RM 113785	Rosthern, Sk	Yes	Yes
AL 19-1203	2003	RM 113848	Saskatoon, Sk	Yes	Yes
AL 20-1203	2003	RM 113886	Rosetown, Sk	Yes	
AL 21-1203	2003	RM 113911	Watrous, Sk	Yes	Yes
AL 22-1203	2003	RM 114005	Sedley, Sk	Yes	
AL 23-1203	2003	RM 114010	Sedley, Sk	Yes	
AL 24-1203	2003	RM 114733	Davidson, Sk		
AL 25-1203	2003	RM 114765	Rosetown, Sk	Yes	Yes
AL 26-1203	2003	RM 114780	Govan, Sk		
AL 27-1203	2003	RM 114889	Landis, Sk	Yes	Yes
AL 28-1203	2003	RM 114908	Watrous, Sk	Yes	Yes
AL 29-1203	2003	RM 115073	Eston, Sk	Yes	Yes
AL 30-1203	2003	RM 115111	Scott, Sk		
AL 31-1203	2003	RM 115144	Landis, Sk	Yes	Yes
AL 32-1203	2003	RM 115163	Dinsmore, Sk	Yes	Yes
AL 33-1203	2003	RM 115493	Laird, Sk		
AL 34-1203	2003	RM 115581	Kenaston, Sk	Yes	
AL 35-1203	2003	RM 115624	Beechy, Sk	Yes	Yes
AL 36-1203	2003	RM 115673	Saskatoon, Sk		
AL 37-1203	2003	RM 115846	Kenaston, Sk	Yes	Yes
AL 38-1203	2003	RM 115965	Wilke, Sk	Yes	
AL 39-1203	2003	RM 116000	Beechy, Sk	Yes	
AL 40-1203	2003	RM 116367	Handel, Sk	Yes	
AL 41-1203	2003	RM 116702	Beechy, Sk	Yes	
AL 42-1203	2003	RM 116957	Davidson, Sk	Yes	
AL 43-1203	2003	RM 116957	Eston, Sk	Yes	
AL 6-0901	2001	unknown	Success, Sk	Yes	
AL 01-1104	2004	unknown	unknown	Yes	
AL 02-1104	2004	unknown	unknown	Yes	
AL 4-1104	2004	unknown	unknown	Yes	
AL 5-1104	2004	unknown	unknown	Yes	

Figure1: Average disease reaction of 31 isolates of *Ascochyta lentis* from pre 1985 and 33 from post 2000 measured as Area Under the Disease Progress Curve relative to the standard isolates AL60901 on 16 lentil genotypes.

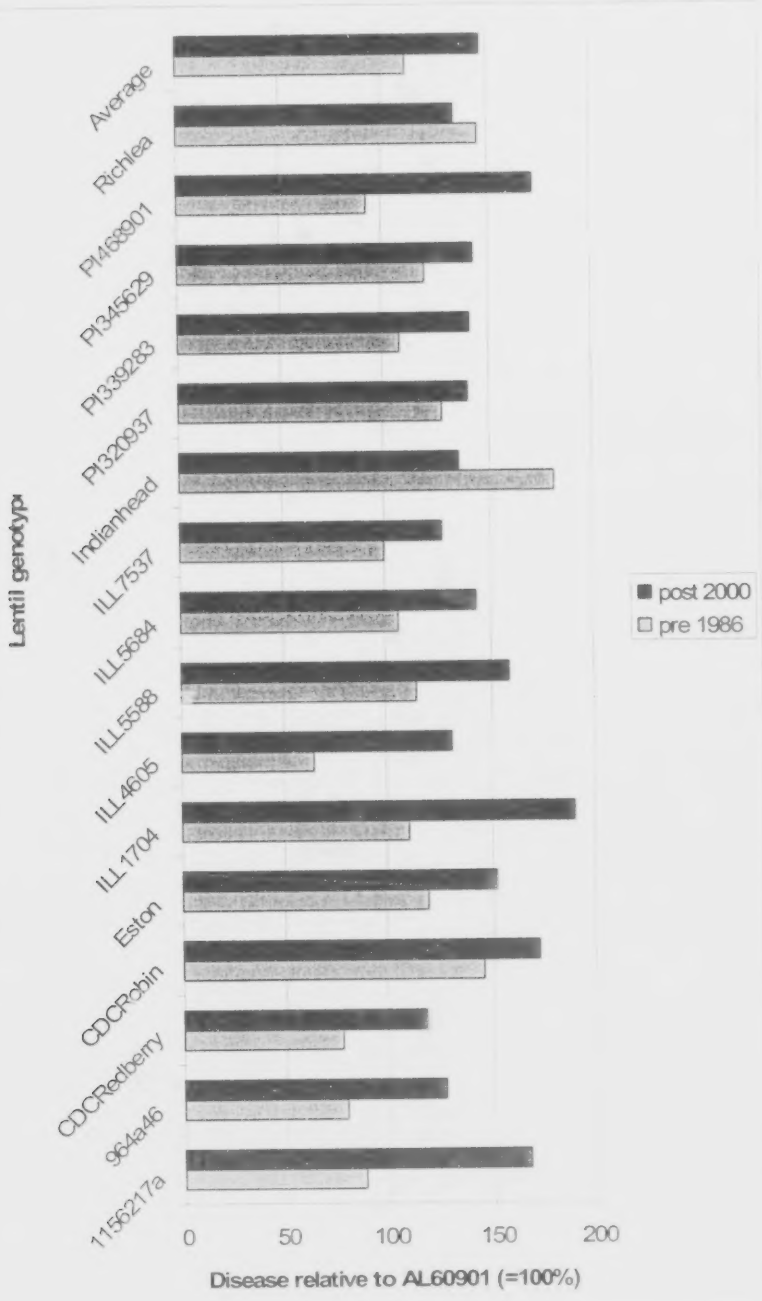

Table 2: Sums of square, contribution of factors to the total variation, and significance levels of ANOVA-type analyses of disease data (measured as Area Under the Disease Progress Curve) of 65 isolates of *Ascochyta lentis* tested on 16 lentil genotypes in 9 tests.

		ANOVA			ANOVA-type statistic[1]			
		DF	SS	% of total SS	DF_d [2]	DF_n [3]	*F* value	*P* > *F*
Test 1	Total	575	4571903					
	iso	8	470410	10	7.55	211	15.74	<.0001
	var	15	1335732	29	12.9	211	24.47	<.0001
	iso*var	120	1305963	29	63.8	211	2.89	<.0001
Test 2	Total	575	4474661					
	iso	8	579038	13	7.78	173	47.77	<.0001
	var	15	1476599	33	13.2	173	52.04	<.0001
	iso*var	120	1107234	25	53.4	173	4.25	<.0001
Test 3	Total	575	125997					
	iso	8	24998	20	7.62	221	40.22	<.0001
	var	15	31161	25	13.9	221	27.18	<.0001
	iso*var	120	34965	28	67	221	3.69	<.0001
Test 4	Total	447	2128915					
	iso	6	516478	24	5.9	147	81.32	<.0001
	var	15	669903	31	12.9	147	43.61	<.0001
	iso*var	90	520877	24	44.5	147	4.78	<.0001
Test 5	Total	575	4255221					
	iso	8	802484	19	7.07	163	52.35	<.0001
	var	15	1761441	41	12.8	163	93.46	<.0001
	iso*var	120	867809	20	50.6	163	2.64	<.0001
Test 6	Total	575	10109150					
	iso	8	2182904	22	7.2	184	62.03	<.0001
	var	15	4093240	40	13	184	72.09	<.0001
	iso*var	120	1965732	19	56.5	184	4.55	<.0001
Test 7	Total	575	5695113					
	iso	8	1290806	23	7.53	198	75.01	<.0001
	var	15	1582730	28	12.3	198	72.19	<.0001
	iso*var	120	1561800	27	60	198	3.59	<.0001
Test 8	Total	447	1517796					
	iso	6	371090	24	5.23	167	63.05	<.0001
	var	15	374367	25	14.1	167	21.17	<.0001
	iso*var	90	332760	22	49.8	167	2.63	<.0001
Test 9	Total	319	2821631					
	iso	4	169265	6	3.66	92.6	10	<.0001
	var	15	1070888	38	9.39	92.6	31.11	<.0001
	iso*var	60	461644	16	26.4	92.6	2.2	0.003

[1] ANOVA-type statistic based on an approximate F distribution (Schah and Madden, 2004)

[2] Degree of freedom denominator

[3] Degrees of freedom numerator

Figure 2: Disease reaction (measured as Area Under the Disease Progress Curve, AUDPC) of 65 isolates (31 from pre 1982, 34 from post 2000) of *Ascochyta lentis* on 16 lentil genotypes

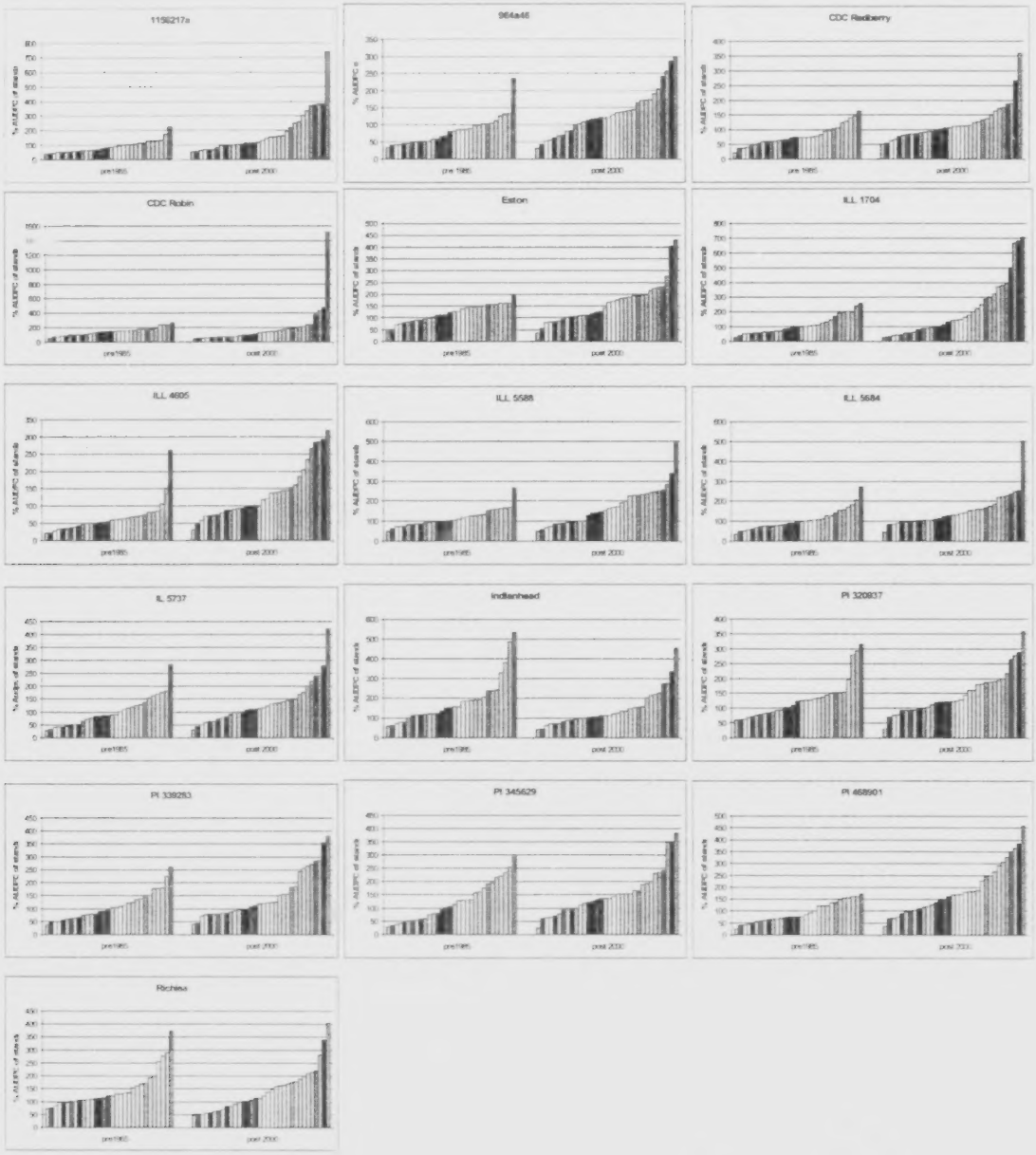

Figure 3: Disease severity (measured as Area Under the Disease Progress Curve, AUDPC) of 6 isolates of *Ascochyta lentis* tested on 16 lentil genotypes. Isolates with blue bars are from pre 1985, those with red bars from post 2001. Note: Direct comparison between isolates is not possible as every isolates was screened in a different test.

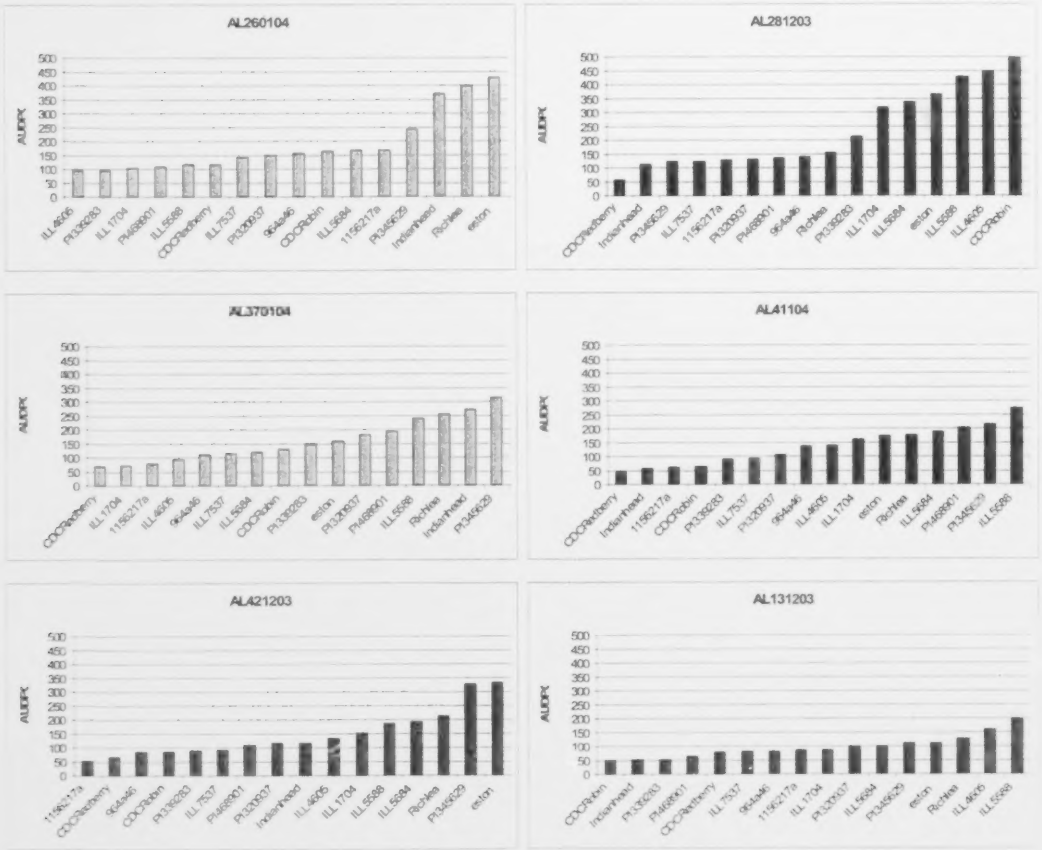

Figure 4: Polymorphism in banding pattern after RAPD-PCR using primer OP Y03 among isolates of *Ascochyta lentis*

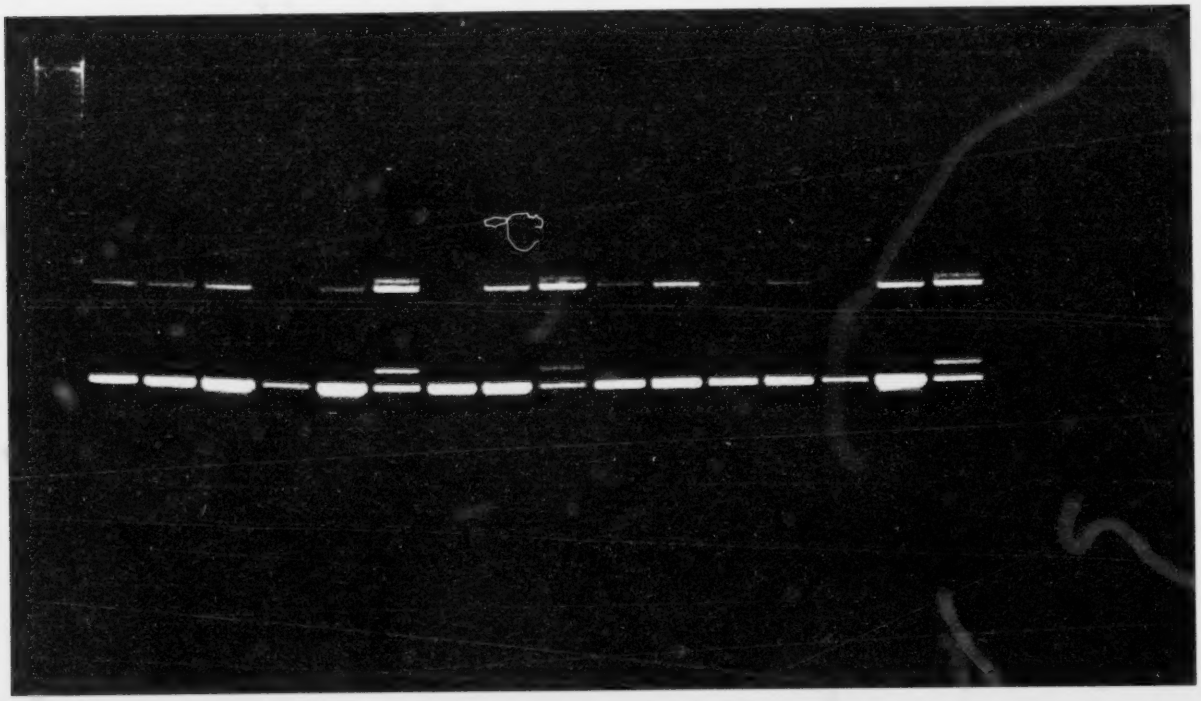

Figure 5: Dendogram displaying similarities between 34 isolates of *Ascochyta rabiei* (blue = pre 1986, red = post 2000) based on RAPD-PCR using 8 Operon primers. Cluster analysis was based on Dice's similarity index using the unweighted group pair method with average (UPGMA).

Conclusions:

Isolates of *A. lentis* were obtained from several years reaching back to 1978. Comparing these different populations revealed that isolates on average have become more aggressive. The degree of increase in aggressiveness varied from lentil genotype to genotype. ILL5588, one of two important sources of resistance to the pathogen appeared to be susceptible to the majority of isolates suggesting that they have adapted to the resistance genes in that genotype. Good resistance was observed in Indianhead which is the second major source of resistance employed in the lentil breeding program. However, two isolates were identified that cause more disease on Indianhead than all or almost all other lentil genotypes tested. Similarly, one isolate was identified that caused most disease on CDC Robin that is supposed to have good resistance to ascochyta blight. These isolates will need to be re-tested to confirm these reactions, but suggest that biotypes are present in the population of *A. lentis* that can overcome these sources of resistance.

The distribution of disease reactions of isolates on all genotypes, and of all isolates on each genotype represented continia of increasing disease severity suggesting a quantitative nature of aggressiveness in the pathogen and of resistance in lentil. However, cross-over reactions of isolates on specific lentil lines, e.g. Indianhead and ILL5588 suggest that major genes may be involved in aggressiveness in the pathogen and resistance in lentil.

Molecular analysis of isolates revealed high levels of similarity (> 80 %) among isolates collected more than 20 years apart. However, isolates from different time periods grouped together. Some isolates were identified with identical banding patterns that originated from different years or different locations, suggesting that isolates may persist in the population in time and space.

The data obtained from this study represents benchmark data for future monitoring efforts of the population of *A. lentis*.

Papers/Presentations:

McHale, S., Cho, C., Vandenberg, A., and **S. Banniza**, 2005: Investigation into the population structure of *Ascochyta lentis*, causal agent of ascochyta blight on lentil.

Manuscripts will be prepared once the entire project has been completed.

Future Research:

This project was confounded by ADF and the Saskatchewan Pulse Growers and will be completed in April. Until then we will screen more isolates and primers to strengthen the molecular analysis of isolates. We will also retest those isolates that showed particular patterns on the lentil genotypes to confirm these results. The results from this project represent benchmark data for future monitoring studies of the pathogen population. Evaluation of aggressiveness in the pathogen and resistance in the crop plant are necessary at certain intervals in time to insure that resistance employed in breeding programs is still effective in the field.

Information of benefit to producers, processors, or governments:

Ascochyta lentis, the fungus that causes ascochyta blight on lentil has become more aggressive. One major sources of resistance seems to have lost its effectiveness against the pathogen.

(c) **Personnel:**

Technical support was supplied by Parvaneh Hashemi who has been employed on the project. In-kind contribution was provided by Stephanie McHale.

(e) Equipment:

None

(f) Project Developed Materials:

None to date

(g) Project Photos:

Can be provided upon request

(h) Acknowledgement:

The Saskatchewan Agricultural Development Fund has been acknowledged in all presentations where this research project was mentioned.